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THE ROLE OF GROWTH HORMONE IN CARBOHYDRATE METABOLISM

BY

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THE ROLE OF GROWTH HORMONE IN CARBOHYDRATE METABOLISM*

BY

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THE ROLE OF GROWTH HORMONE IN CARBOHYDRATE METABOLISM*

The relationship of the anterior pituitary gland to carbohydrate metabolism first was shown by the work of Houssay.[1,2,3] He demonstrated that removal of the hypophysis ameliorated the disturbances induced in the dog by pancreatectomy and, furthermore, that administration of anterior lobe extracts produced hyperglycemic, glycosuria, and ketonuria in normal dogs. Since Long[4] could not produce glycosuria and ketosis with anterior pituitary extracts in the absence of the adrenal glands, he suggested that the anterior lobe extracts exert their diabetogenic activity, at least in some degree, via the adrenal cortex. These findings differed from those reported by Houssay and Leloir,[5] *viz.*, the diabetes produced by anterior pituitary extracts in the dog persisted even after removal of the adrenal glands. However, it should be noted that such a diabetic state produced by pituitary extracts is not easily reversed by any procedure. At present, it cannot be denied that the adrenocorticotrophic hormone (ACTH) of the anterior pituitary has a potent diabetogenic action in both normal animals and men.[6]

The present paper is concerned with studies undertaken to determine (1) whether or not an anterior pituitary factor(s) other than ACTH has a role in the regulation of normal carbohydrate metabolism, and if so; (2) to determine its identity; (3) its mode of action; and (4) its relationships to the other hormonal agents.

In the investigations that are reported, two approaches were employed. In the first, various hormonal deficiency states were produced so as to provide indirect evidence as to the importance of an anterior pituitary factor (other than ACTH) in carbohydrate metabolism. Second, various replacement therapies were employed in order to ascertain the relative significance of each of the anterior pituitary hormones in this problem. The various hormonal deficiency states studied were those of (a) the adrenal-ectomized; (b) adrenalectomized-gonadectomized; and (c) adrenalectomized-hypophysectomized dogs in regard to their carbohydrate metabolism. The various replacement therapies consisted of administration of either anterior pituitary hormones or hormones of the target glands.

Methods

All experiments were carried out on trained unanesthetized male and female, normal, adrenalectomized, adrenalectomized-gonadectomized, hypophysectomized, and adrenalectomized-hypophysectomized dogs,

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Adrenalectomy* was performed as a two-stage operation. Adrenalectomy and gonadectomy were performed simultaneously, i.e., one gonad was removed at the same time as the homolateral adrenal gland and, thus, two-stage gonadectomy proceeded concurrently with two-stage adrenalectomy. The technique of hypophysectomy,[7] and the after-care[8] of the hypophysectomized dogs have been described in previous papers. The hypophysectomized dogs used in this study manifested a high degree of sensitivity (60-100x that of normal dogs) to the hypoglycemic action of insulin.[8,9] The adrenalectomized-hypophysectomized dogs were prepared by a three-stage operation; i.e., either an adrenalectomized dog was subsequently hypophysectomized or an hemi-adrenalectomized dog was hypophysectomized and then had its other adrenal removed. All the dogs used in these investigations were fed a mixed diet[8] and, in addition, the adrenalectomized, adrenalectomized-gonadectomized, and adrenalectomized-hypophysectomized dogs received a daily maintenance of minimal doses (1.5-2.5 mg. intramuscularly) of desoxycorticosterone acetate (DCA) in oil (Schering), and thereby were maintained in good condition. Experiments were performed in animals that ate regularly and that had consumed their full food allotment on the day preceding the experiment. All experiments were undertaken in the post-absorptive state, 17 to 18 hours after the last feeding.

Insulin sensitivity was determined by the response to 0.025 unit/kg. insulin (Lilly) administered intravenously (referred to as the "test dose") as well as to 0.25 unit/kg. insulin intravenously (10x the test dose); blood sugar changes were followed for 4 hours. Blood sugar was determined by a modified Hagedorn-Jensen[10] method using the Somogyi acid-zinc filtrate.[11] The insulin sensitivity of the adrenalectomized and adrenalectomized-gonadectomized dogs was tested while on DCA maintenance and again during DCA-free periods lasting 8 to 14 days, i.e., until signs of adrenocortical insufficiency appeared. Several dogs were subjected to a number of such DCA-free periods. Serum sodium concentrations (measured on an internal standard flame photometer) and non-protein nitrogen (NPN) determinations (using the micro-Kjeldahl technique) were made while each animal was on DCA and repeated during the DCA-free period. Remedial measures were carried out in the dogs manifesting signs of severe adrenal insufficiency only after the last insulin sensitivity test had been performed. Then, these dogs were given saline and glucose infusions together with intravenous injections of desoxycorticosterone glucoside (DCG, Ciba)[2] and hydrocortisone (free alcohol, Merck).[2] The dogs also were placed on their daily DCA maintenance. The glucose tolerance was determined by following the blood sugar changes over a period of 4 hours after completion of a 10 minute intravenous infusion, 0.075 g./kg./min. glucose.

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* We should like to express our appreciation to: Dr. R. K. Richards of Abbott Laboratories for Pentothal Sodium, Nembutal, and Penicillin G Procaine Aqueous; Dr. A. C. Bratton, Jr. of Parke, Davis and Company for Thrombin Topical and Penicillin S-R; Drs. E. Henderson and P. L. Perlman of Schering Corp. for Cortate; Dr. K. K. Chen of Eli Lilly and Company for Insulin and Duracillin; Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc. for desoxycorticosterone glucoside; Dr. E. Alpert of Merck and Company for Hydrocortone acetate and Hydrocortone (free alcohol); Drs. E. E. Hays and I. M. Bunding of Armour Laboratories for growth hormone; Dr. R. W. Bates, formerly of E. R. Squibb and Sons, for prolactin; and Dr. K. W. Thompson of Roche-Organon, Inc. for thyroxine.

Most of the growth hormone preparations used in these studies were Armour[2] preparations: lots #22KR1, #J21609R, and #GH 3. In addition, a growth hormone preparation supplied by Dr. C. H. Li was used. The dosages of these various growth hormone regimens ranged from 0.02-1.5 mg./kg./day given intramuscularly once daily after the afternoon feeding. The dosage, duration and circumstances of administration of any one growth hormone regimen in any specific experimental dog will be described below. In one group of experiments on hypophysectomized dogs, the adrenocortical steroids, cortisone acetate or hydrocortisone acetate[2] were given in daily dosages of 0.83-1.2 mg./kg. intramuscularly in 2 divided doses (9 A.M., 5 P.M.) for periods of 17 to 38 days at which time growth hormone administration (Armour preparation #J21609R) was started. Growth hormone, 1.0-1.5 mg./kg. intramuscularly, was given once daily, usually after the afternoon feeding, for 12 to 31 days in conjunction with adrenocortical steroids. The dosage and duration of the combined steroid-growth hormone regimens in any particular hypophysectomized dog are given below in detail. Thyroxine (Roche-Organon),[2] 0.25-0.52 mg./kg./day intramuscularly, was given to hypophysectomized dogs for 7 to 16 days. Some hypophysectomized dogs received whole thyroid, 600 mg./day for 8 to 10 days. Prolactin, Squibb preparation #71713, was given to hypophysectomized dogs in dosages of 1.0-5.0 mg./kg./day intramuscularly for as long as 30 days.

Results

1. *Responses to insulin and intravenous glucose of adrenalectomized, adrenalectomized-gonadectomized and adrenalectomized-hypophysectomized dogs.* If it is indeed true that some anterior pituitary factor(s) other than ACTH has a role in normal carbohydrate metabolism, then the adrenalectomized dog, with its intact pituitary but inoperative ACTH,* should not have as disturbed a carbohydrate metabolism as the adrenalectomized-hypophysectomized dog which, in addition to the lack of ACTH, is completely deficient in all the other anterior pituitary hormones.

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* Experiments carried out on adrenalectomized dogs showed that their responses to insulin were unaffected by ACTH regimens.

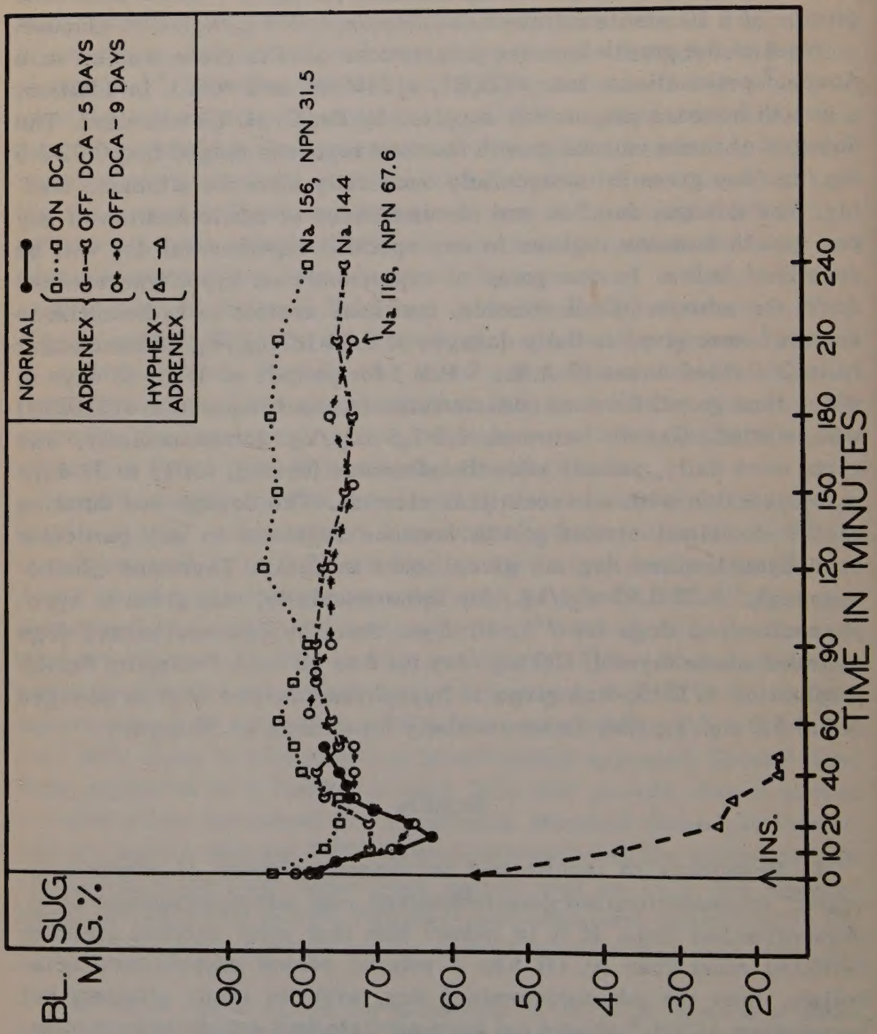

FIGURE 1 *(For description see facing page)*

In the first phase of these investigations, the responses to insulin, 0.025 u./kg. i.v. (the test dose), of several adrenalectomized dogs were studied and then compared with the insulin responses of both normal dogs and adrenalectomized-hypophysectomized dogs (FIGURES 1 and 2). The insulin sensitivity tests were first performed while both the adrenalectomized and adrenalectomized-hypophysectomized dogs were maintained on minimal daily doses of desoxycorticosterone acetate (DCA, 1.5-2.5 mg. i.m.). Although two types of responses were obtained with the test dose of insulin in the adrenalectomized dogs; *viz.*, one group of 6 dogs revealed little or no change in their insulin responses from that of normal dogs, and the other group of 3 dogs had a significantly greater insulin sensitivity than normal dogs; nevertheless, all the adrenalectomized dogs were far less sensitive to the test dose of insulin than the adrenalectomized-hypophysectomized dogs in which a most striking insulin hypersensitivity was observed. It should be noted that all adrenalectomized dogs (including those minimally sensitive to 0.025 unit/kg. insulin) manifested a marked sensitivity much greater than normal to 10x the test dose of insulin, 0.25 unit/kg. i.v. This was in agreement with previously reported work in which this dose of insulin was used.[12]

To eliminate whatever effect DCA may have had upon the insulin responses of the adrenalectomized dogs, all these animals were subjected to DCA-free periods of varying duration until signs of severe adrenal insufficiency had supervened; *viz.*, anorexia, vomiting, apathy, low serum sodium levels, and abnormally elevated blood NPN levels. FIGURE 1 records the insulin responses of adrenalectomized dog K-48 during a DCA-free period. Dog K-48 represents that group of dogs the insulin responses of which were similar to those of normal dogs. Although the serum sodium fell to 116 mEq. and the NPN rose to 67.6 mg.% during a DCA-free period of 9 days, it can be seen that the insulin response remained unchanged from that obtained during the DCA maintenance period.

FIGURE 2 records the insulin responses of dog K-52, one of 3 dogs that had a much greater sensitivity to insulin than normal dogs. They, too, were subjected to DCA-free periods and, again, no significant changes in the responses to insulin were observed during the DCA-free period so long as the animals were tested in the post-absorptive state. This was true although a declining serum Na level and rising blood

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FIGURE 1. *(see opposite page)* Blood sugar curves produced by insulin (0.025 u./kg. i.v.) in adrenalectomized (Adrenex) dog K-48 compared with those of normal and hypophysectomized-adrenalectomized (Hyphex-Adrenex) dogs. The curves of dog K-48 represent its responses to insulin while on DCA maintenance and again during a DCA-free period.

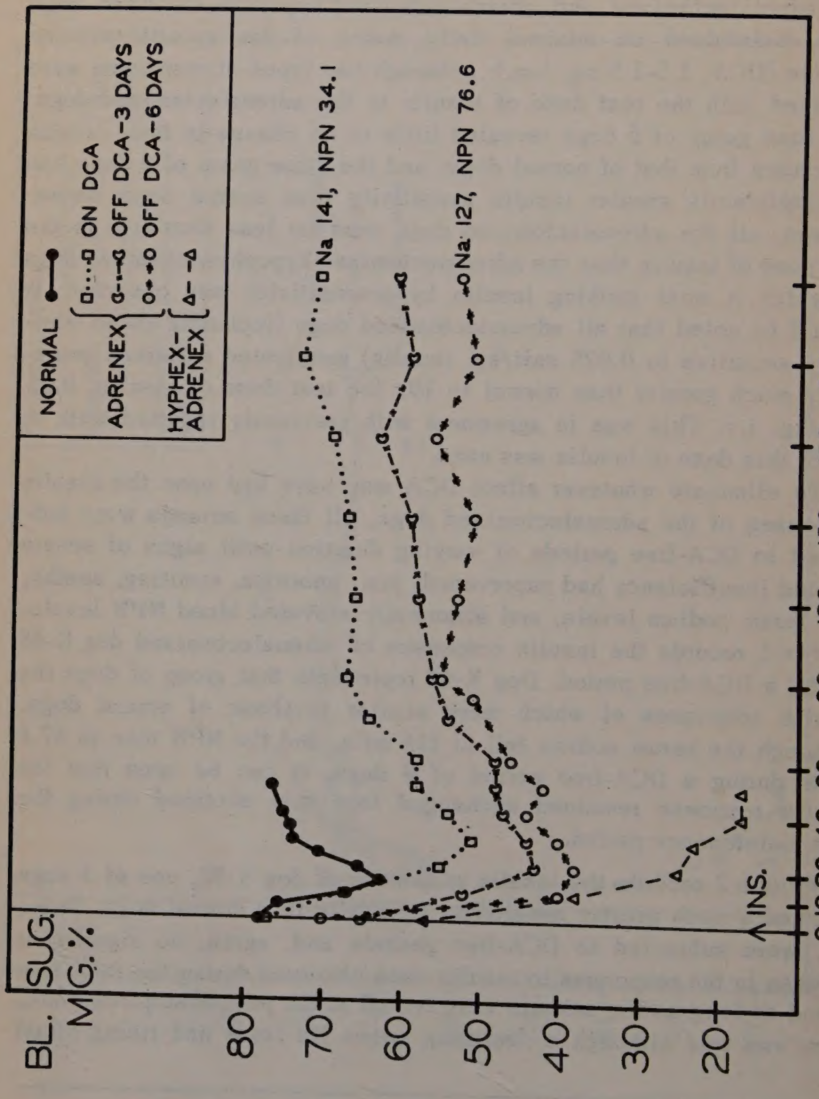

FIGURE 2 *(For description see facing page)*

NPN, together with the other signs previously mentioned, indicated the existence of a state of adrenal insufficiency.

Although the adrenalectomized dogs, free from any administered DCA, showed signs of adrenal insufficiency but still revealed no greater insulin sensitivity than had been observed on DCA maintenance, the question was posed as to whether or not the adrenalectomized dogs' sex steroids might not compensate for the lack of the adrenal steroids in carbohydrate metabolism, although obviously unable to significantly affect their electrolyte metabolism.

As shown in FIGURE 3, the response to insulin of adrenalectomized-gonadectomized dogs was only slightly greater than that observed in normal dogs, and this was even less than that observed in several of the adrenalectomized non-gonadectomized dogs. From a comparison with the insulin response of adrenalectomized-hypophysectomized dogs, it is evident that the adrenalectomized-gonadectomized dogs were much less sensitive to insulin than the former animals.

FIGURE 4 records a composite insulin response of all adrenalectomized and adrenalectomized-gonadectomized dogs compared with both normal and adrenalectomized-hypophysectomized dogs. The absence of some anterior pituitary hormone(s), other than ACTH, as an important factor responsible for the difference in responses is clearly evident.

The second phase of these studies concerned the responses to intravenous glucose of adrenalectomized, adrenalectomized-gonadectomized and adrenalectomized-hypophysectomized dogs. FIGURE 5 indicates the striking difference between the composite responses to intravenous glucose of adrenalectomized and adrenalectomized-hypophysectomized dogs. Although the glucose tolerance of the adrenalectomized dogs shows a more rapid fall in the blood sugar than that of normal dogs and a secondary hypoglycemia, the glucose tolerance of adrenalectomized-hypophysectomized dogs has a greatly accelerated rate in the fall of the blood sugar and a much more severe secondary hypoglycemia which at times results in convulsions. Again, the adrenalectomized-gonadectomized dogs, in their responses to intravenous glucose, showed no difference from those of the adrenalectomized dogs, i.e., the fall in the blood sugar occurred at about the same rate and there was a secondary hypoglycemia of the same degree. This provides further evidence for the greater derangement in carbohydrate metabolism of the adrenalectomized-hypophysectomized animal.

FIGURE 2. *(see opposite page)* Blood sugar curves produced by insulin (0.025 u./kg. i.v.) in adrenalectomized (Adrenex) dog K-52 compared with those of normal and hypophysectomized-adrenalectomized (Hyphex-Adrenex) dogs. The curves of dog K-52 represent its responses to insulin while on DCA maintenance and again during a DCA-free period.

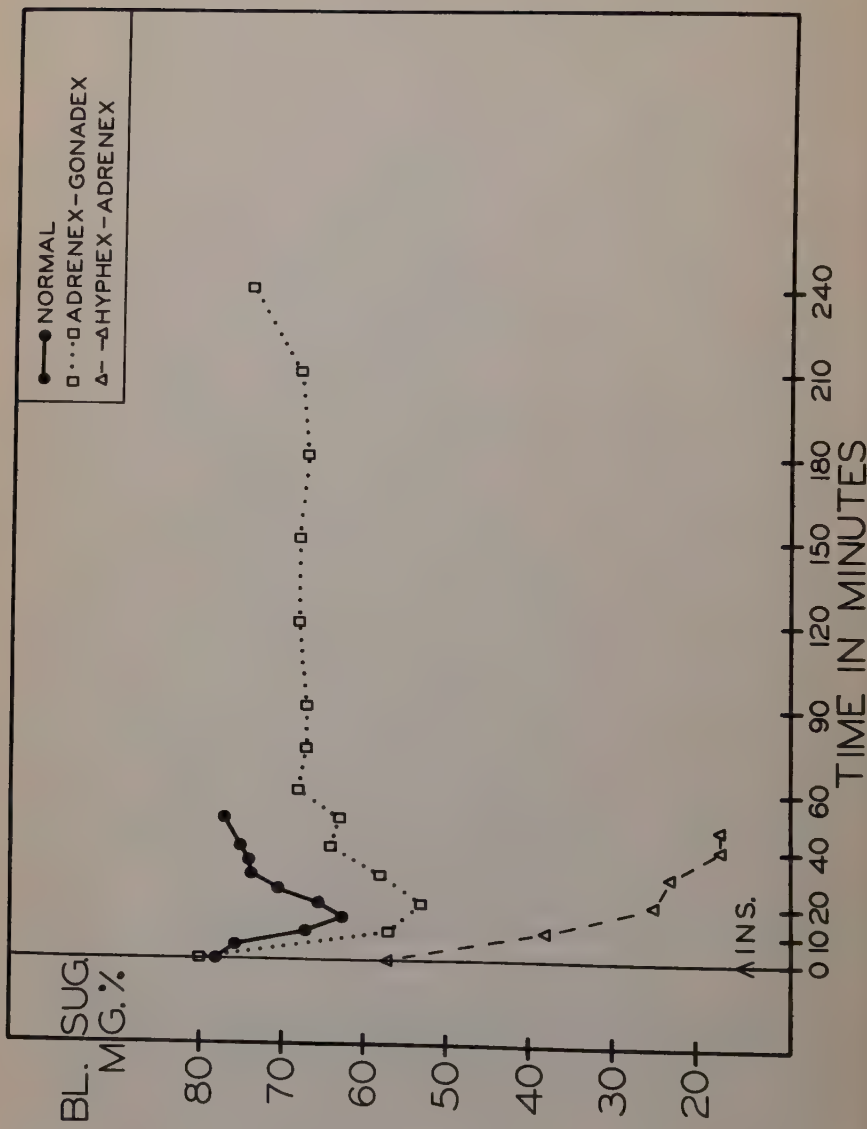

FIGURE 3 *(For description see facing page)*

Comment. From a comparison of the responses to insulin and intravenous glucose of adrenalectomized and adrenalectomized-hypophysectomized dogs, it is obvious that the anterior pituitary gland elaborates hormone(s) other than ACTH which participate in the regulation of carbohydrate metabolism. Furthermore, comparison of these responses of the adrenalectomized and adrenalectomized-gonadectomized dogs reveals that the gonadotrophins cannot be implicated as a factor in the regulation of carbohydrate metabolism, inasmuch as these two types of dogs had similar responses to insulin and intravenous glucose.

It must be concluded then that some other anterior pituitary factor(s) was responsible for the difference in carbohydrate metabolism of the adrenalectomized and adrenalectomized-hypophysectomized dogs. In addition, this factor(s) must have been able to exert effects on carbohydrate metabolism without the presence of either the adrenal or sex steroids.

2. *Effects of thyroxine (thyroid) and prolactin on carbohydrate metabolism of hypophysectomized dogs.* Since the above experiments admittedly offered only indirect evidence for the importance of some anterior pituitary hormone(s), it was decided to investigate the effects of the anterior pituitary hormones other than ACTH and the gonadotrophins on the insulin response and glucose tolerance of hypophysectomized dogs.

The first experiments utilizing this approach were performed with the intention of assessing the importance of the thyrotrophic factor of the anterior pituitary. Thyrotrophin itself was not used in the hypophysectomized dogs since the preparations available were impure and there existed the possibility that the effects of other trophic factors, present in the preparation, might confuse the results obtained. Rather, several hypophysectomized dogs were placed on either whole thyroid or thyroxine regimen in dosages that eventually were found to produce a state of clinical hyperthyroidism; *viz.*, irritability, increased heart rate, elevated body temperature, loss of body weight, *etc.* The response to the test dose of insulin, 0.025 unit/kg. i.v., was used as the measure of the effect of thyroxine or thyroid on the carbohydrate metabolism of these animals, FIGURE 6 records the responses of dog #3 given thyroxine, 0.25-0.52 mg./kg. i.m., once daily, for 16 days, during which time the animal lost 1.6 kg. body weight, developed a heart rate of 180, had tremors, and was extremely restless. It can be seen that there was some anti-insulin action exerted by thyroxine.

FIGURE 3. *(see opposite page)* Blood sugar curves produced by insulin (0.025 u./kg. i.v.) in adrenalectomized-gonadectomized (Adrenex-Gonadex) dogs compared with those of normal and hypophysectomized-adrenalectomized (Hyphex-Adrenex) dogs.

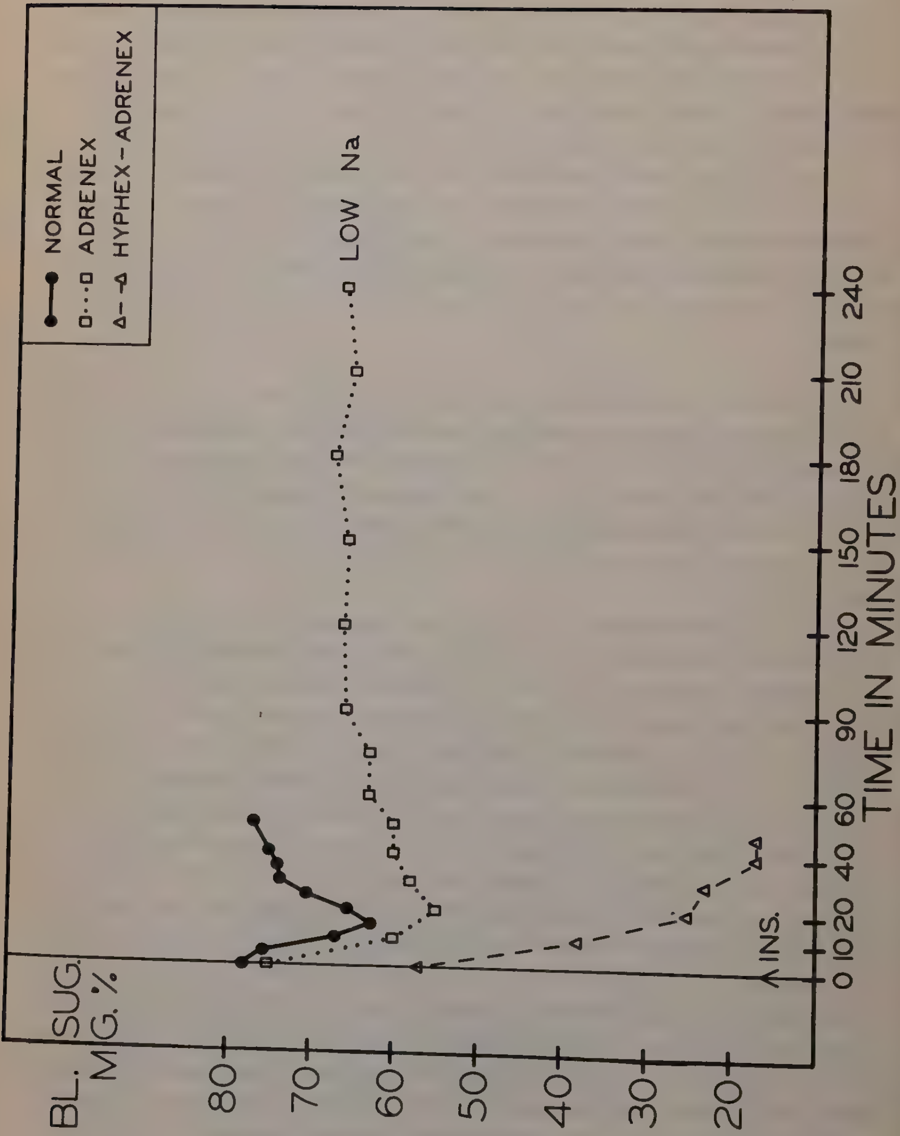

FIGURE 4 *(For description see facing page)*

This action was not marked, however, and its significance was considered dubious since a state of hyperthyroidism was produced. In other hypophysectomized dogs, the thyroxine dosage was administered for periods of 7 to 9 days, during which time all the dogs showed signs of hyperthyroidism; yet, the effect of thyroxine on the insulin responses of these animals was negligible. Other hypophysectomized dogs were placed on regimens of 600 mg. thyroid per day for 8 to 10 days until signs of hyperthyroidism were in evidence but, again, the effect upon the sensitivity to insulin was negligible. Therefore, the anterior pituitary factor being sought did not appear to be the thyrotrophic factor in view of the above results obtained in hypophysectomized dogs.

With the exclusion of both thyrotrophin and the gonadotrophins, attention next was directed to prolactin, or the lactogenic factor. Squibb preparation #71713 was used in dosages of 1.0 mg./kg./day, given intramuscularly once daily. Its ACTH content was estimated to be, at most, about 2 per cent. Furthermore, its growth hormone content certainly was less than 5 per cent, inasmuch as prolactin #71713 produced no growth in hypophysectomized rats when given in daily dosages of 1 to 5 mg., whereas growth hormone preparations produced appreciable growth in similar hypophysectomized rats in daily dosages of 0.02 to 0.05 mg. In some hypophysectomized dogs, 3 daily doses of prolactin produced a partial abolition of their insulin hypersensitivity. With both a prolongation and increase in the dosage regimens, varied effects were obtained. In some dogs, the anti-insulin effect became larger, whereas in others, the anti-insulin action did not become any more marked. The latter effect of prolactin is shown in Table 1 in which the response to insulin of hypophysectomized dog #85 are recorded after 7 and 14 doses of prolactin, 1.0 mg./kg./day. As can be seen, no appreciable change in the anti-insulin effect occurred. With continuation of the therapy for 30 days and an increase in dosage up to 3.0 mg./kg./day and then 5.0 mg./kg./day, the response to insulin of dog #85 remained the same as it has been after 7 and 14 days of prolactin (table 1). At the same time, the response to intravenous glucose of the hypophysectomized dogs showed no tendency towards diabetes throughout the course of the prolactin regimens.

Comment. There is no doubt that prolactin #71713 exerted an anti-insulin effect in the hypophysectomized dog. The ACTH contaminant was considered to be rather small and probably not responsible for the

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Figure 4. *(see opposite page)* Composite blood sugar curves produced by insulin (0.025 u./kg. i.v.) in normal, hypophysectomized-adrenalectomized (Hyphex-Adrenex) dogs, and all adrenalectomized (Adrenex) dogs during a DCA-free period.

above action. It was known from previous work that potent ACTH regimens were required in order to obtain complete abolition of the insulin hypersensitivity of hypophysectomized dogs.[13,14] Although this cast serious doubt that the ACTH could have produced such effects in these experiments, the possibility as to its being the causative factor was not completely eliminated. Likewise, although prolactin #71713 had no growth activity, even in dosages of 5.0 mg./kg./day, the exact amount of its growth hormone contaminant could not be clearly determined. Its having less than 5 per cent growth hormone was at best only a calculated approximation. In view of these considerations, the importance of prolactin in carbohydrate metabolism is still open to question and awaits further elucidation.

TABLE 1

RESPONSES TO THE TEST DOSE OF INSULIN
OF HYPOPHYSECTOMIZED (HYPHEX) DOG #85
BEFORE AND DURING TREATMENT WITH PROLACTIN
COMPARED WITH THAT OF NORMAL DOGS

Time in Min.	Normal Composite*	Hyphex Dog #85			
	Daily Doses of Prolactin**				
	0	0	7***	14°	30+
	Blood Sugar in mg.%				
0	78	67	79	78	76
Insulin 0.025 u./kg. intrav. at 0 time					
10	67	51	61	63	67
20	66	36	50	46	44
30	74	32	46	43	46
40	75	37	59	52	51
50	77	37	64	58	55
60		40	66	68	59
75		39	73	65	64
90		47	74	71	70
120		52	81	79	75
150		59	76	76	76
180		61	77	-	77
210		65	73	81	78
240		64	73	81	80

* Based on 37 insulin sensitivity tests on 18 normal dogs.

** Squibb prolactin #71713 used.

*** Dose: 1 mg./kg. i.m.

° Dose: 1 mg./kg. i.m.

+ Dose: 1 mg./kg. i.m. for 14 days; 3 mg./kg. i.m. for 12 days
5 mg./kg. i.m. for 4 days.

3. *Anti-insulin and diabetogenic actions of growth hormone in hypophysectomized dogs.* Previous work reported from this laboratory has shown that growth hormone (Armour #22KR1) regimens, in dosages of 1.0 mg./kg./day, could completely abolish the insulin hypersensitivity and secondary hypoglycemia of the glucose tolerance test of hypophysectomized dogs.[8,15,16] These regimens, however, also produced diabetes and insulin resistance. In addition, such toxic signs as anorexia, vomiting and weakness were observed, which at times resulted in death. Thus, growth hormone exerted marked anti-insulin and diabetogenic effects in these animals. Similar results were obtained with other Armour growth hormone preparations in comparable dosages

TABLE 2

INSULIN SENSITIVITY AND GLUCOSE TOLERANCE TESTS
IN NORMAL AND HYPOPHYSECTOMIZED (HYPHEX) DOGS
BEFORE AND DURING GROWTH HORMONE (GH) LI TREATMENT

(A) Insulin Sensitivity Tests				(B) Glucose Tolerance Tests			
	Normal Composite*	Hypophysectomized Dog K-78			Normal Composite°	Hyphex Composite**	Hyphex Dog K-78
	Doses of GH+				Doses of GH+		
Time in Min.	0	0	4	Time in Min.	0	0	5
	Blood Sugar in mg.%				Blood Sugar in mg.%		
0	78	70	85	0	80	65	94
Insulin 0.025 u./kg. intrav. at 0 time				Glucose 0.075 g./kg./min. i.v. for 10 min.			
10	67	54	77	10	274	329	360
20	66	39	78	15	208	276	308
30	74	35	75	35	97	177	242
40	75	34	76	55	80	120	200
50	77	38	81	70	82	89	190
60		45	81	85	81	74	187
75		49	81	100	79	64	181
90		46	84	115	81	59	176
120		48	86	130	78	54	170
150		52	86	160	84	50	162
180		58	86	190	84	51	154
210		67	85	220	84	53	146
240		58	86	250	83	57	137

* Based on 37 insulin sensitivity tests on 18 normal dogs.

° Based on 7 glucose tolerance tests on 7 normal dogs.

** Based on 11 glucose tolerance tests on 10 hypophysectomized dogs.

+ Growth hormone supplied daily by Dr. C. H. Li. Dose: 1 mg./kg./day i.m. once daily.

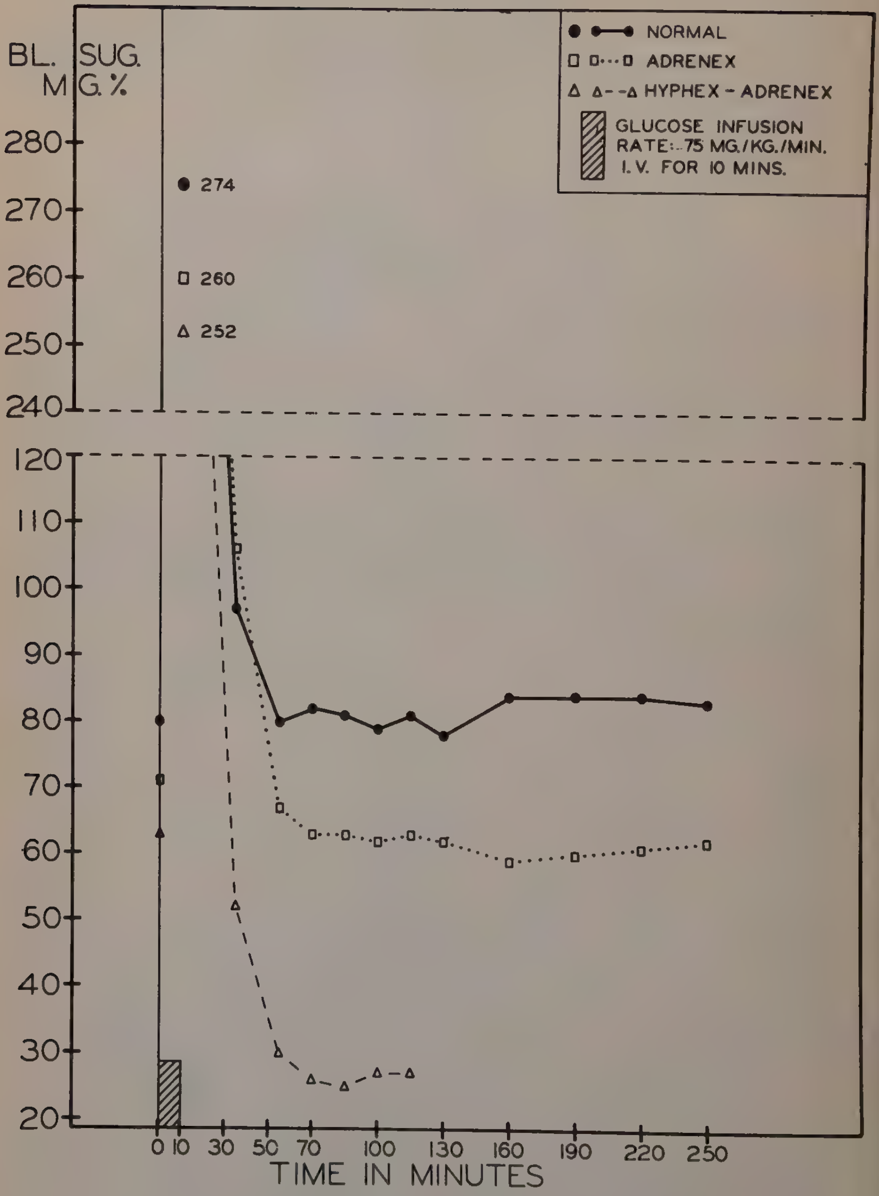

FIGURE 5 *(For description see facing page)*

as well as with a growth hormone preparation kindly supplied by Dr. C. H. Li (TABLE 2, dog K-78). Other investigators reported the production of diabetes with growth hormone in both normal[17,18,19] and subtotally depancreatized[20] dogs and cats. This diabetogenic action, nevertheless, can be considered to be of physiological significance only if it could be demonstrated that it represented an exaggeration of a truly physiological action of the hormone in carbohydrate metabolism. It thus became essential to demonstrate that growth hormone can produce a partial or complete abolition of the insulin hypersensitivity of the hypophysectomized dog without the production of a concomitant diabetes, when given in proper dosages and under proper conditions. If this effect were achieved and could be continued indefinitely, only then could the growth hormone be considered to have a physiological role in carbohydrate metabolism.

With this in mind, the dosage regimens of Armour growth hormone preparation #22KR1 were reduced to 9.3 mg./kg./day intramuscularly. TABLE 3, dog K-73, shows that continued administration of growth hormone in this dosage produced complete abolition of the insulin hypersensitivity of the hypophysectomized dogs with no change in the glucose tolerance. Insulin resistance was not observed. Similarly, a regimen of Armour growth hormone preparation #GH 3 (20 to 30 per cent as potent as #22KR1 as shown by rat bioassay) was given in dosages of 1 mg./kg./day intramuscularly and produced abolition of the insulin hypersensitivity of the hypophysectomized dogs without the concurrent production of diabetes or insulin resistance. No evaluation of a prolonged administration of these growth hormone regimens could be made since they induced toxic states which dictated a withdrawal of therapy from the animals so treated.

A further lowering of the daily dosage of the growth hormone regimens was instituted. Armour growth hormone preparation #J21609R was used, the potency of which was about 75 per cent of that of Armour #22KR1. It was given in dosages of 0.02 mg./kg./day intramuscularly, which was the minimal dose with which noticeable anti-insulin effects could be observed (TABLE 4). Even during these minimal dose regimens, however, 50 per cent of the hypophysectomized dogs still developed a tendency towards diabetes, as seen, for example, on the glucose tolerance test of dog K-39 (TABLE 4). Of interest was the fact that prolonged administration (for more than 40 days in all cases) was possible without the appearance of any toxic manifestations. This possibly

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FIGURE 5. *(see opposite page)* Composite blood sugar curves produced by intravenous glucose (0.75 gm./kg.) in normal, adrenalectomized (Adrenex), and hypophysectomized-adrenalectomized (Hyphex-Adrenex) dogs.

could be related to a concurrent diminution in the anti-insulin effect of this growth hormone regimen after a few weeks (refractoriness to growth hormone?). Indeed, the animals, while on growth hormone, eventually reverted to the insulin sensitivity of their untreated state. Concomitant renal function studies,[*] performed during the periods when the anti-insulin effects were evident, revealed no change from that

TABLE 3

INSULIN SENSITIVITY AND GLUCOSE TOLERANCE TESTS
IN NORMAL AND HYPOPHYSECTOMIZED (HYPHEX) DOGS
BEFORE AND DURING TREATMENT WITH ARMOUR GROWTH HORMONE (GH)
#22KR1

(A) Insulin Sensitivity Tests

	Normal Composite*	Hypophysectomized Dog K-73	
	Doses of GH+		
Time in Min.	0	0	8
	Blood Sugar in mg.%		
0	78	57	92
Insulin 0.025 u./kg. intrav. at 0 time			
10	67	37	81
20	66	30	72
30	74	28	75
40	75	28	79
50	77	27	83
60		28	85
75		26	86
90		27	87
120		31	87
150		32	
180		33	
210			
240			

(B) Glucose Tolerance Tests

	Normal Composite°	Hyphex Composite**	Hyphex Dog K-73
	Doses of GH+		
Time in Min.	0	0	7
	Blood Sugar in mg.%		
0	80	65	80
Glucose 0.075 g./kg./min. i.v. for 10 min.			
10	274	329	334
15	208	276	258
35	97	177	166
55	80	120	126
70	82	89	98
85	81	74	79
100	79	64	70
115	81	59	61
130	78	54	54
160	84	50	58
190	84	51	56
220	84	53	63
250	83	57	81

[*] Based on 37 insulin sensitivity tests on 18 normal dogs.

[°] Based on 7 glucose tolerance tests on 7 normal dogs.

[**] Based on 11 glucose tolerance tests on 10 hypophysectomized dogs.

[+] Dose of growth hormone: 0.3 mg./kg. body weight intramuscularly once daily.

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[*] These renal function studies were done in collaboration with Drs. D. P. Earle and S. J. Farber, Department of Medicine, New York University College of Medicine.

seen during the untreated state. This was in marked contrast to the effect of growth hormone regimens (#J21609R and #22KR1), 1 mg./kg./day, on the renal functions of hypophysectomized dogs.[21,22] In these dosages they markedly elevated the glomerular filtration rate and renal plasma flow of these dogs.

TABLE 4

INSULIN SENSITIVITY AND GLUCOSE TOLERANCE TESTS
IN NORMAL AND HYPOPHYSECTOMIZED (HYPHEX) DOGS
BEFORE AND DURING TREATMENT WITH SMALL DOSES OF GROWTH HORMONE (GH)

(A) Insulin Sensitivity Tests				
	Normal Composite*	Hypophysectomized Dog K-39		
	Doses of GH+			
Time in Min.	0	0	9	14
	Blood Sugar in mg.%			
0	78	61	75	76
Insulin 0.025 u./kg. intrav. at 0 time				
10	67		63	67
20	66	36	47	50
30	74	32	42	47
40	75	32	41	46
50	77		43	47
60		32	47	48
75			48	51
90		36	49	45
120		45	59	48
150			63	49
180		52	60	55
210			66	57
240		55	68	62

(B) Glucose Tolerance Tests			
	Normal Composite°	Hypophysectomized Dog K-39	
	Doses of GH+		
Time in Min.	0	0	18
	Blood Sugar in mg.%		
0	80	72	80
Glucose 0.075 g./kg./min. i.v. for 10 min.			
10	274	290	404
15	208	264	334
35	97	175	167
55	80	118	132
70	82	78	108
85	81	56	91
100	79	47	82
115	81	44	69
130	78	46	63
160	84	50	58
190	84	54	57
220	84	57	57
250	83	60	58

* Based on 37 insulin sensitivity tests on 18 normal dogs.

° Based on 7 glucose tolerance tests on 7 normal dogs.

+ Dose of growth hormone; 0.02 mg./kg. body weight intramuscularly once daily.

Comment. Since the minimal doses of growth hormone, administered for very long periods without the production of toxic signs, still gave evidence for a tendency towards diabetes in a large number of the hypophysectomized dogs, it is most probable that, with the other dosage regimens cited above, a diabetic-type of glucose tolerance

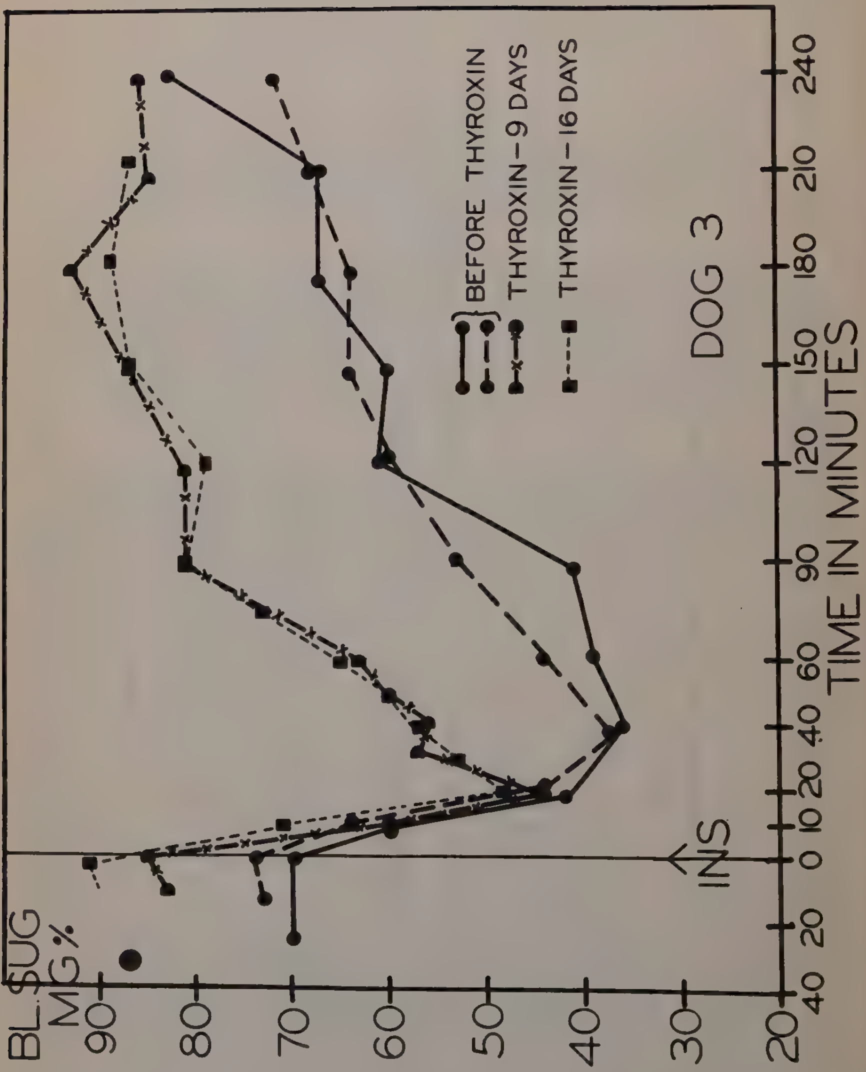

FIGURE 6 *(For description see facing page)*

would have been observed had it been possible to continue the regimens for a longer period of time. This was not possible because of the development of one or more signs of toxicity with each of the several regimens.

4. *Effects of combined adrenocortical steroid-growth hormone regimens in hypophysectomized dogs.* In a previous report from these laboratories, it was shown that growth hormone regimens, 1 mg./kg./day, could restore the depressed renal functions of hypophysectomized dog partially towards normal [21,22] (FIGURE 7). More recent studies have demonstrated that continued administration of either ACTH [23] or adrenocortical steroids,[24] too, can elevate the renal functions of such dogs but only partially towards normal (FIGURE 7). In view of these findings, hypophysectomized dogs were placed on a combined adrenocortical steroid-growth hormone regimen, and concurrent studies were carried out to determine the effects of such regimens on the carbohydrate metabolism of these dogs.

In all the dogs, the adrenocortical steroids first were administered alone. Either cortisone or hydrocortisone regimens, 0.83-1.2 mg./kg./day, were used. Continued administration of the steroids (1) abolished the insulin hypersensitivity of the hypophysectomized dogs (FIGURE 8, dog K-44) without the production of insulin resistance; (2) abolished the secondary hypoglycemia of the glucose tolerance with no marked elevation in the peak or a shift to the right in the decline of the blood sugar (FIGURE 9, dog K-44); and (3) restored the response to intravenous adrenaline to normal. When the steroid regimens had produced a normal carbohydrate metabolism in the hypophysectomized dogs (as judged by the above criteria), growth hormone (Armour #J21609R), 1 mg./kg./day, was given in conjunction with the steroid regimens.

After receiving a compound F regimen, 0.83 mg./kg./day for 17 days, dog K-44 was given growth hormone, 1.0 mg./kg./day, and the steroid administration was continued. After 11 days on such a combined hormonal regimen, the response to insulin of dog K-44 was no different from that observed during steroid therapy alone (FIGURE 8). Likewise, the glucose tolerance of dog K-44, when tested after 5 and 22 days on the combined hormonal regimen (the 22nd and 39th days, respectively, of compound F therapy), remained unaltered from that obtained during the steroid regimen alone. Indeed, this was true even when the growth hormone dosage was increased from 1.0 mg./kg./day to 1.5 mg./kg./day (FIGURE 9). Similarly, the adrenaline response remained at normal levels.

FIGURE 6. *(see opposite page)* Blood sugar produced by insulin (0.025 u./kg. i.v.) in hypophysectomized dog No. 3 before and during a thyroxine regimen. The thyroxine regimen consisted of 0.025-0.52 mg./kg./day i.m.

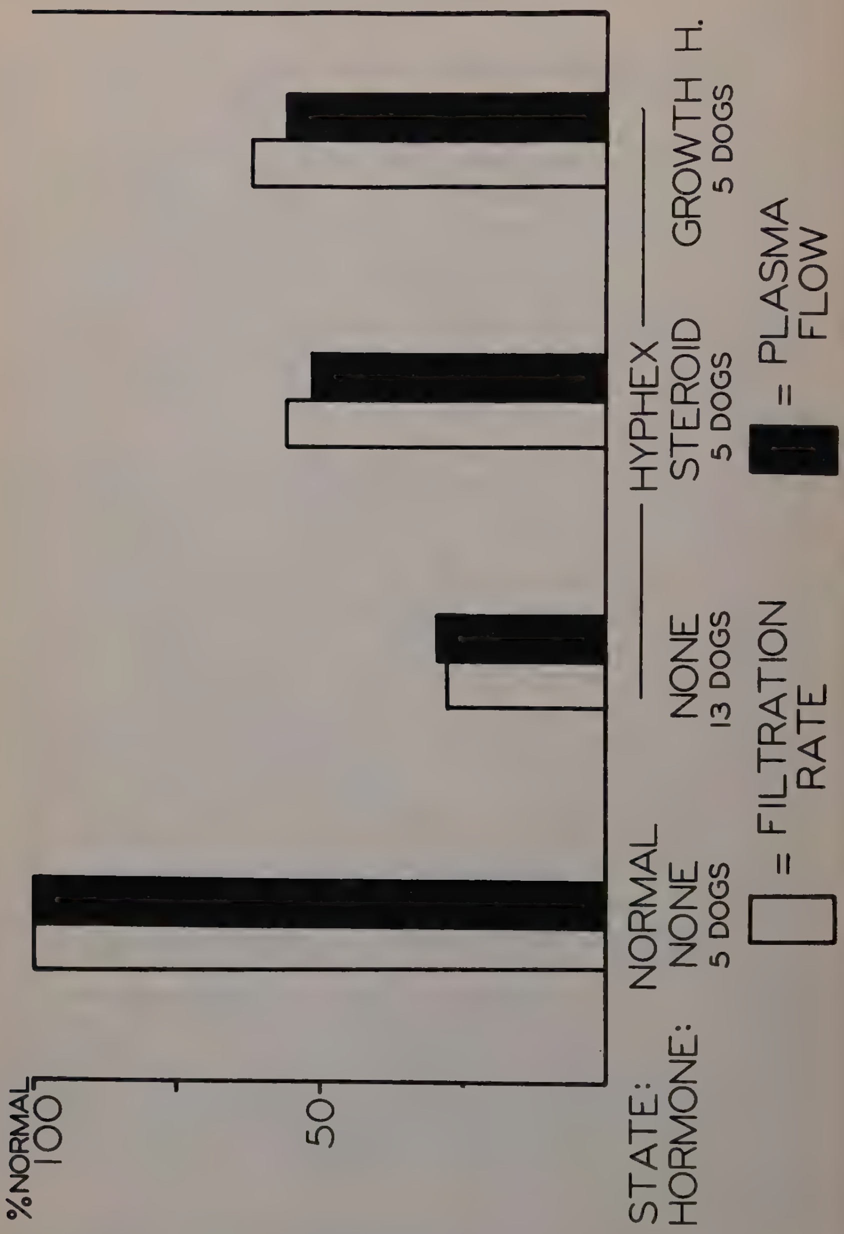

FIGURE 7 *(For description see facing page)*

Since there was apparently a restoration to normal in the carbohydrate metabolism of these dogs, a larger dose of insulin (0.025 unit/kg. i.v. = 10x the test dose) was used to determine whether or not any of the animals had developed insulin resistance during the combined hormonal regimen. As seen from a typical experiment shown in FIGURE 10 (dog K-44), the animals still responded like normal dogs. This was in marked contrast to previous findings with growth hormone in which insulin resistance was produced even to doses of insulin as large as 1.5 unit/kg. intravenously.[8]

There was no doubt that the growth hormone (Armour #J21609R) used in the above experiments was a potent, "diabetogenic" preparation. When given to untreated hypophysectomized dogs it produced (1) abolition of their insulin sensitivity (FIGURE 11, dog K-80), (2) a diabetic state (FIGURE 12, dog K-80), and (3) toxic signs such as anorexia, vomiting and lethargy. Additional evidence for the marked activity of Armour #J21609R was obtained from the renal function studies performed on the hypophysectomized dogs given the combined adrenocortical steroid-growth hormone regimens. When growth hormone therapy was given in conjunction with the adrenal steroids, the renal functions of these dogs were elevated to normal from those levels obtained with the steroid regimens alone (FIGURE 13). After growth hormone had been discontinued, the animals receiving only steroids, the renal functions fell to those levels previously obtained during the steroid regimens alone. Thus, the rise and fall in renal functions could be attributed to the addition or removal, respectively, of an active growth hormone preparation.

Comment. An analysis of the results obtained with combined adrenocortical steroid-growth hormone regimens reveals the following salient features: (1) restoration to normal of the disturbed carbohydrate metabolism of hypophysectomized dogs was achieved with steroid regimens alone; (2) this restoration was not altered by the diabetogenic action of growth hormone; and (3) neither a summation nor potentiation of the individual effects of these two blood sugar-raising agents (the steroids and growth hormone) eventuated.

Indeed, there was no indication that growth hormone contributed in any way its known anti-insulin effect to the pronounced anti-insulin action of the steroids. Had the steroid levels in these experiments been much smaller, thereby resulting in only a partial restoration of the

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FIGURE 7. *(see opposite page)* Effects of either growth hormone or adrenocortical steroid regimens on the glomerular filtration rate (GFR) and renal plasma flow (RPF) of hypophysectomized (Hyphex) dogs compared with the renal functions before hypophysectomy.

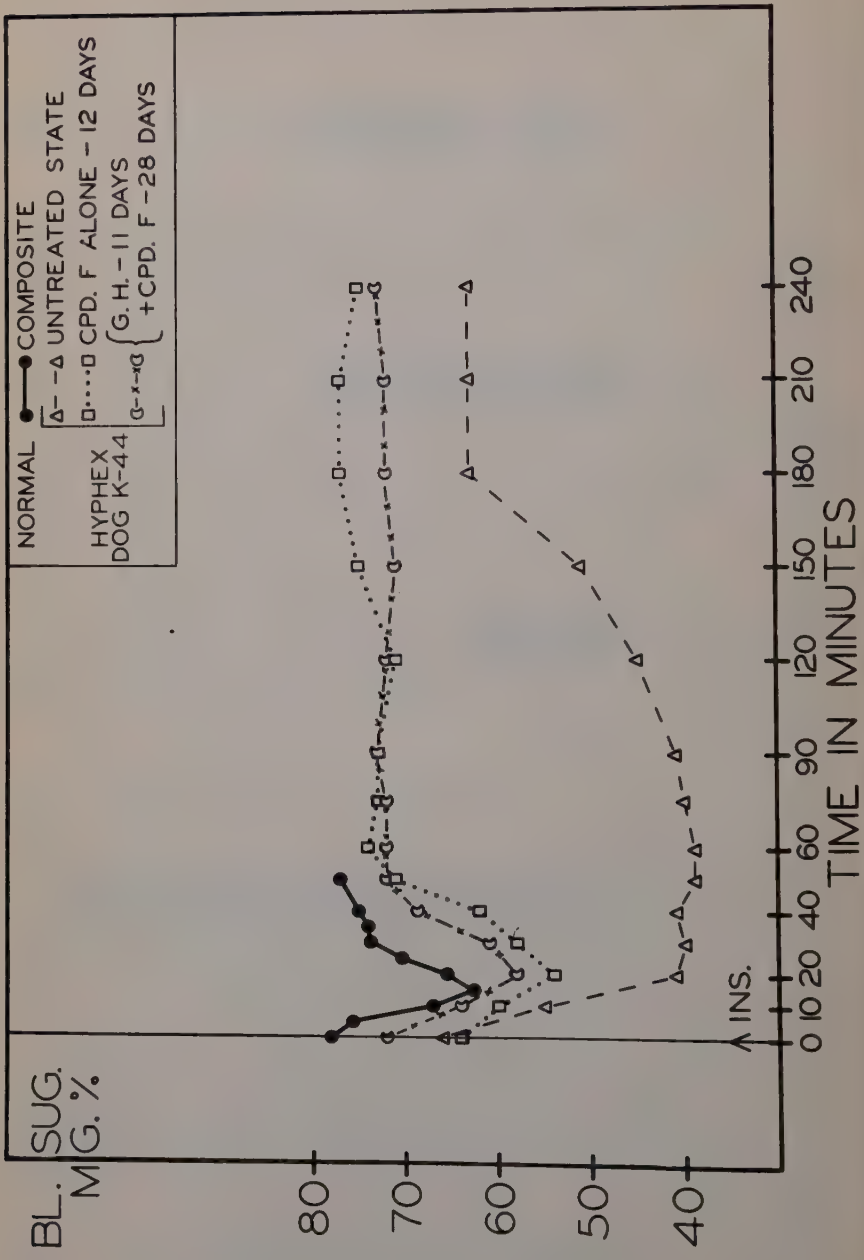

FIGURE 8 *(For description see facing page)*

hypophysectomized dog's carbohydrate metabolism to normal, the anti-insulin action of growth hormone might have become evident. Although the steroid regimens, in the dosages used, had restored the abnormal carbohydrate metabolism of adrenalectomized dogs ostensibly to normal, they were considered above physiological levels since they had elevated the renal functions of the adrenalectomized dogs far above the normal range.

5. *Effects of growth hormone in the adrenalectomized-hypophysectomized dog.* Some type of relationship of growth hormone to the adrenocortical steroids was indicated by the above results. If, however, the insulin responses of adrenalectomized dogs were less than that of adrenalectomized-hypophysectomized dogs, because of the anti-insulin action of growth hormone, it follows that the adrenocortical steroids were not essential for this action. Experiments were performed in which growth hormone was administered to adrenalectomized-hypophysectomized dogs maintained on minimal daily doses of DCA. FIGURE 16 shows a typical result obtained in such an animal, dog K-77. First, it can be seen that the adrenalectomized-hypophysectomized dog manifests a remarkable insulin hypersensitivity and, second, growth hormone Armour #J21609R (1.0 mg./kg./day) produced an equally striking abolition of that sensitivity. It should be noted at this time that growth hormone administration to the adrenalectomized-hypophysectomized dogs had to be halted, too, in view of the onset of signs of toxicity.

Comment. Although this effect occurred in the presence of DCA (2.0 mg./day i.m.), the evidence to date would indicate that DCA has has little, if any, effect in carbohydrate metabolism and cannot potentiate the action of growth hormone. The sex steroids, too, are not necessary for the action of growth hormone if the experiments concerned with the responses to insulin and intravenous glucose of both adrenalectomized and adrenalectomized-gonadectomized dogs are recalled. It is concluded, therefore, that growth hormone does not require the adrenal or sex steroids in order to manifest its anti-insulin and diabetogenic actions. To exert its actions without diabetes and/or toxicity, however, it appears that growth hormone requires the adrenocortical steroids.

FIGURE 8. *(see opposite page)* Blood sugar curves produced by insulin (0.025 u./kg. i.v.) in (a) normal dogs and in hypophysectomized (Hyphex) dog K-44; (b) during the untreated state; (c) during compound F regimen (0.83 mg./kg./day) alone; and (d) during combined compound F-growth hormone regimen (1.0 mg./kg./day). Compound F given for a total of 28 days. Growth hormone Armour No. J21609R begun on the 18th day of compound F regimen and continued for 11 days.

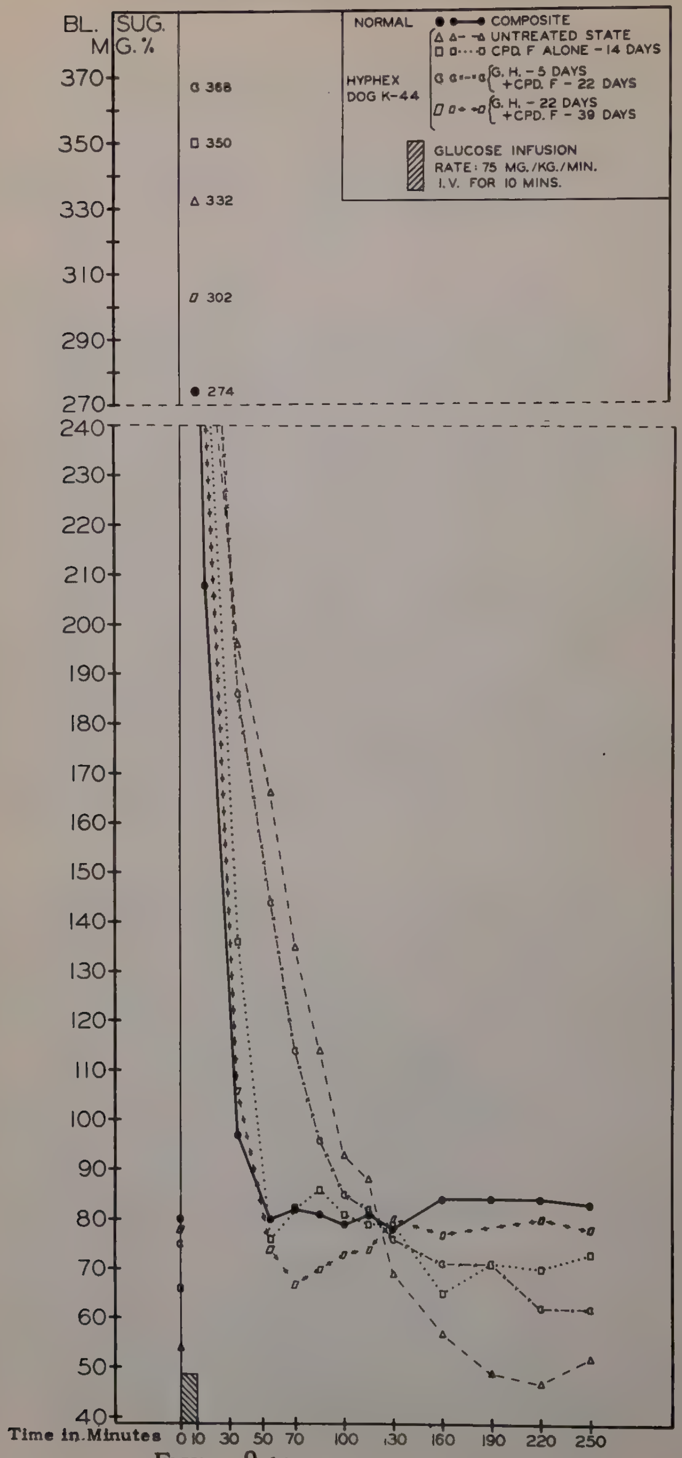

FIGURE 9 *(For description see facing page)*

Discussion

Briefly reviewing the experimental findings, it can be stated that the adrenalectomized dog is much less sensitive to insulin than the adrenalectomized-hypophysectomized dog. It is evident, therefore, that there is an anterior pituitary factor, other than ACTH, that exerts a significant effect in the regulation of carbohydrate metabolism. The gonadotrophins are not implicated since the responses to insulin and intravenous glucose of adrenalectomized and of adrenalectomized-gonadectomized dogs showed no appreciable differences. Likewise, as was shown, the thyrotrophic factor can be eliminated. The importance of prolactin is not yet clarified, but appears to be negligible. Thus, by elimination the anterior pituitary growth hormone remains to be considered.

A great amount of evidence, indeed, is available that growth hormone has an action in carbohydrate metabolism. This has been demonstrated in normal,[17,18,19] subtotally depancreatized,[20] and hypophysectomized animals.[8,15,16] Moreover, it has been shown by the *in vitro* studies on the isolated diaphragm of hypophysectomized rats.[25,26] This action of growth hormone cannot be ascribed to ACTH contamination of the preparations, since anti-insulin and diabetogenic actions also can be obtained in the adrenalectomized-hypophysectomized dog. Therefore, it is most probable that growth hormone, or some hormone closely linked with it,* is the most important anterior pituitary factor other than ACTH that has a role in the regulation of carbohydrate metabolism.

In considering the mechanism of action of growth hormone in carbohydrate metabolism, several factors related to the origin and fate of the blood sugar must be discussed. Growth hormone, by virtue of its nitrogen-retaining ability, lowers the blood amino nitrogen, thereby diminishing gluconeogenesis. There is no evidence that growth hormone enhances glycogen mobilization or inactivates insulin. It appears

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* Recently, Raben and Westermeyer reported that their growth hormone preparation was equal in growth-promoting activity to other preparations but had little, if any, diabetogenic activity (proc. Soc. Exper. Biol. and Med. *80*:83, 1952). It remains for future work to corroborate these findings. Whether the anterior pituitary factor exerting effects on carbohydrate metabolism is the growth hormone, or some other factor closely linked with it, is of little consequence to this discussion. There is still an anterior pituitary factor (other than ACTH) that has a role in carbohydrate metabolism.

FIGURE 9. *(see opposite page)* Blood sugar curves produced by intravenous glucose (0.75 gm./kg.) in (a) normal dogs and in hypophysectomized (Hyphex) dog K-44; (b) during the untreated state; (c) during compound F regimen (0.83 mg./kg./day) alone; and (d) during a combined compound F-growth hormone regimen (1.0-1.5 mg./kg./day). Compound F given for a total of 39 days. Growth hormone Armour No. J21609R, 1 mg./kg./day, begun on the 18th day of compound F regimen and continued for 15 days. Dosage of growth hormone then raised to 1.5 mg./kg./day and given for 7 more days.

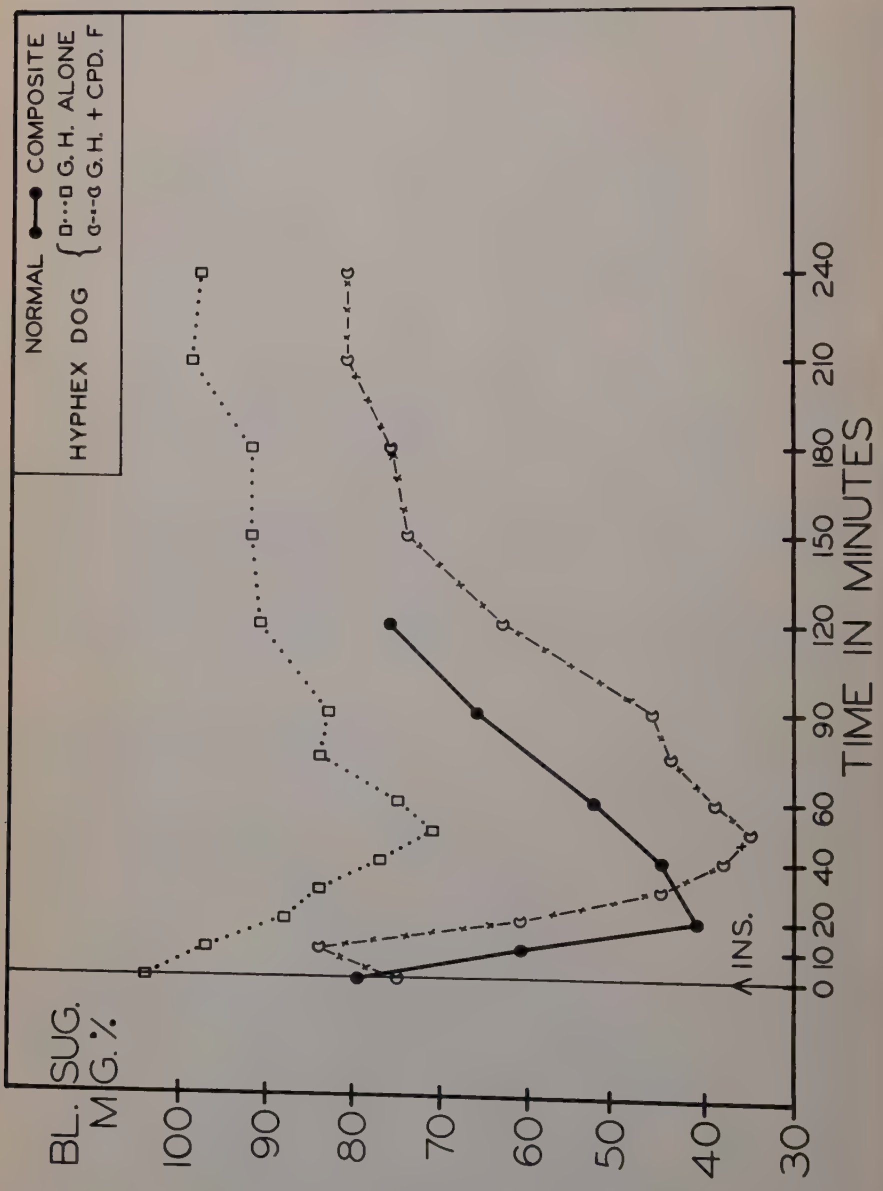

FIGURE 10 *(For description see facing page)*

from the glucose tolerance of growth hormone-treated hypophysectomized dogs that the hormone interferes with the peripheral utilization of sugar. Indeed, the *in vitro* studies of Stadie *et al.*[25] and Park *et al.*[26] on isolated rat diaphragms are in agreement with the conclusions derived from the effects in the living animal. They showed that purified growth hormone decreased the sugar uptake of the isolated rat diaphragm provided that the hormone was injected into the living animal. The action of growth hormone in both the hormone-treated hypophysectomized dog and rat develops only gradually after injection. Purified growth hormone is inactive if added directly to the diaphragm *in vitro*. This suggests that growth hormone possibly liberates another substance during this period after injection, or else undergoes some transformation. In either case, these other factors may be exerting the actions attributed to growth hormone.

It can be stated further on the basis of the above experiments that growth hormone could exert its effects on carbohydrate metabolism without the intervention of either the adrenocortical or sex steroids. This finding appears to be opposed to those reported by Park and Krahl[27] and Stadie.[28] They showed, respectively, that administration of either adrenal cortical extract[27] or cortisone[28] together with growth hormone to hypophysectomized rats was required in order to obtain a growth hormone effect on the glucose uptake by the diaphragms isolated from these rats. The findings of these investigators, moreover, are not only in conflict with our observations but with the earlier reports of Krahl and Cori[29] who found the glucose utilization by diaphragms of adrenalectomized rats to be within the normal range. Furthermore, Stadie[28] also reported previously that adrenalectomy itself had no appreciable effect upon the response of the diaphragm to insulin. If growth hormone needs the steroids for its actions, however, it follows that the diaphragm from adrenalectomized and adrenalectomized-hypophysectomized rats should not differ in their glucose uptakes or responses to insulin, Indeed, there should be no difference *in vivo* between the insulin sensitivity of the adrenalectomized dog and that of the adrenalectomized-hypophysectomized dog. This obviously is not the case since there are demonstrable differences between these two types of animals, both *in vitro* and *in vivo*.

When growth hormone was administered in effective dosages to hypophysectomized dogs, an anti-insulin, or more broadly speaking, a blood sugar-raising action was observed, but toxic manifestations were

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FIGURE 10. *(see opposite page)* Blood sugar curve produced by insulin (0.025 u./kg. i.v. equals 10x the test dose) in normal dogs compared with those of hypophysectomized dogs (a) during a growth hormone regimen (1.0 mg./kg./day) alone and (b) during combined compound F (0.83 mg./kg./day)-growth hormone regimen.

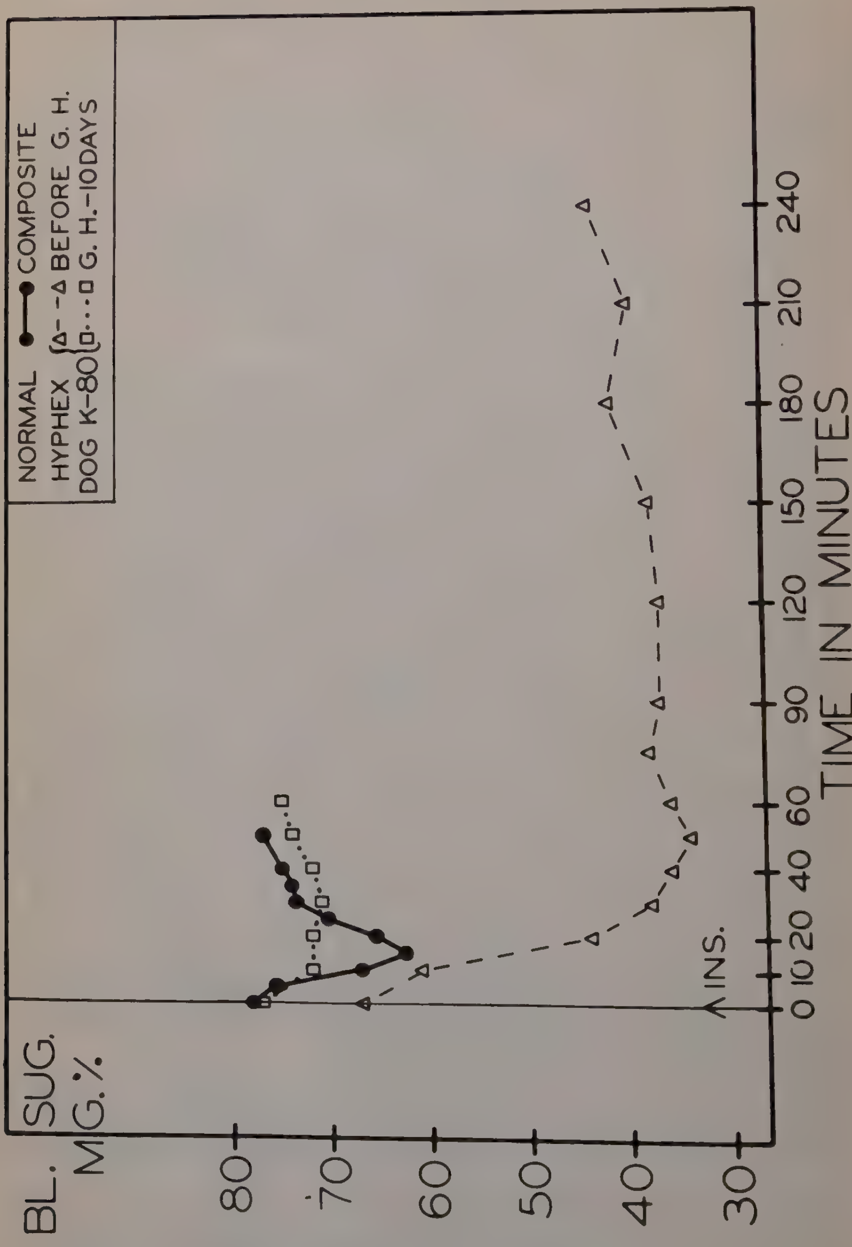

FIGURE 11 *(For description see facing page)*

produced. In physiological life, growth hormone obviously does not produce any toxic effects. The hypophysectomized dog, however, not only lacks growth hormone but has many other hormonal deficiencies. Thus, the hypophysectomized dog does not represent the normal dog lacking only growth hormone. Conversely, the administration of growth hormone alone to hypophysectomized dogs does not fully restore their hormonal status to normal. The experiments in which combined adrenocortical steroid-growth hormone regimens were used provide a clue as to the relationship between growth hormone and other endocrine agents. In these experiments, prolonged administration of growth hormone was made possible without the appearance of any toxic manifestations so long as the adrenocortical steroids were given in conjunction with the growth hormone. Furthermore, the carbohydrate metabolism of the animals, having been restored to normal on steroid regimens alone, was not aggravated by the addition of the growth hormone regimen; i.e., no diabetes or insulin resistance was produced. The combined effects of the adrenal steroids and growth hormone resulted in neither a summation nor a potentiation of their individual effects. Thus, it is presumed that, in some way, a well-adjusted balance prevails between growth hormone and the adrenal steroids in the regulation of carbohydrate metabolism. The explanations thus far offered on the mechanism of action of these hormones cannot explain the findings obtained on combined hormonal regimens. The adrenocortical steroids exert an antagonism to insulin primarily by their effect on gluconeogenesis and, in addition, perhaps by their effect on peripheral utilization of sugar. Growth hormone, too, has a blood sugar-raising action by virtue of its ability to inhibit the peripheral utilization of sugar, and is undoubtedly more diabetogenic than the steroids. Since neither a summation nor a potentiation of the respective effects of adrenocortical steroids and growth hormone eventuated, it is implied that, in some way, the steroids antagonized the growth hormone actions. This antagonism may be due to (1) the inhibition of the secretion and/or action of another substance, the liberation of which is under the influence of growth hormone, or (2) the blocking of a transformation of growth hormone to a more active form, the effects of which are attributed to growth hormone itself. Although it is well-known that these two hormones are antagonistic in growth and nitrogen metabolism,[30,31] as yet no such antagonism has been observed in their action on carbohydrate metabolism.

FIGURE 11. *(see opposite page)* Blood sugar curve produced by insulin (0.025 u./kg. i.v.) in normal dogs compared with those of hypophysectomized (Hyphex) dog K-80 before and during growth hormone regimen. Growth hormone Armour No. J21609R, 1 mg./kg./day, given for 10 days.

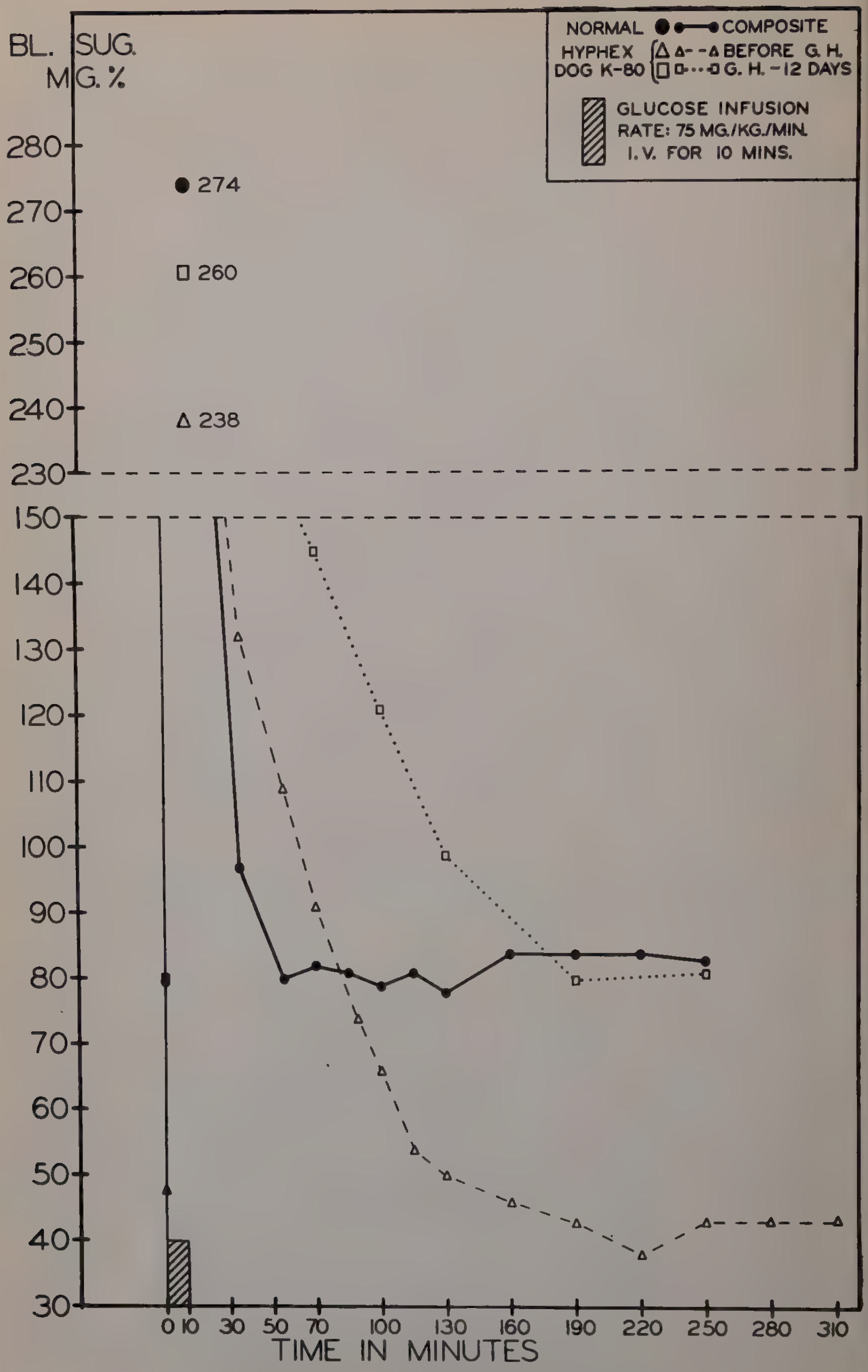

FIGURE 12 *(For description see facing page)*

From the above results, it is concluded that growth hormone is the most important anterior pituitary factor, other than ACTH, in the regulation of carbohydrate metabolism. Its role in this respect is to inhibit the peripheral utilization of sugar, thereby antagonizing the action of insulin and helping to maintain the blood sugar in fasting. Furthermore, its actions in carbohydrate metabolism appear to be in balance with the actions of other endocrine agents, among them the adrenal steroids and insulin.

In addition to the anti-insulin action of growth hormone, it has been demonstrated that, under certain circumstances, growth hormone can produce a hypoglycemia. An intravenous injection of growth hormone to the 17 to 18 hours fasted hypophysectomized dog, for the first time, produces a marked hypoglycemia.[32] If successive daily intravenous injections (all given in the post-absorptive state) are administered, the hypoglycemic action of growth hormone progressively diminishes, so that by the third injection, there is no evidence for any hypoglycemia at all. This may be attributed in part to the development of the anti-insulin action of growth hormone.[32] This hypoglycemic action has not been demonstrated in normal dogs, although the dose was varied from 0.3 to 50 mg./kg. intravenously. It has been reported, however, in both normal and adrenalectomized rats.[33] Furthermore, it does not appear to be related to the pancreas, since it can be demonstrated in depancreatized dogs, too.[32] It does not seem to be related to the protein-synthetizing action of growth hormone, but rather to a transient effect in increasing the peripheral utilization of sugar by diaphragms from hypophysectomized rats.[34] In view of the divergency of some of these findings, the significance of the growth hormone induced-hypoglycemia is still obscure.

SUMMARY AND CONCLUSIONS

1. The adrenalectomized dog, while more sensitive to insulin than the normal dog, is far less sensitive than the adrenalectomized-hypophysectomized dog.
2. The secondary hypoglycemia seen on the glucose tolerance test of the adrenalectomized-hypophysectomized dog is much more severe than that of the glucose tolerance of the adrenalectomized dog.
3. The responses to insulin and intravenous glucose of the adrenalectomized-gonadectomized dog are similar to those of the adrenalectomized dog.

FIGURE 12. *(see opposite page)* Blood sugar curve produced by intravenous glucose (0.75 gm./kg.) in normal dogs compared with those of hypophysectomized (Hyphex) dog K-80 before and during growth hormone regimen. Growth hormone Armour No. J21609R, 1 mg./kg./day, given for 12 days.

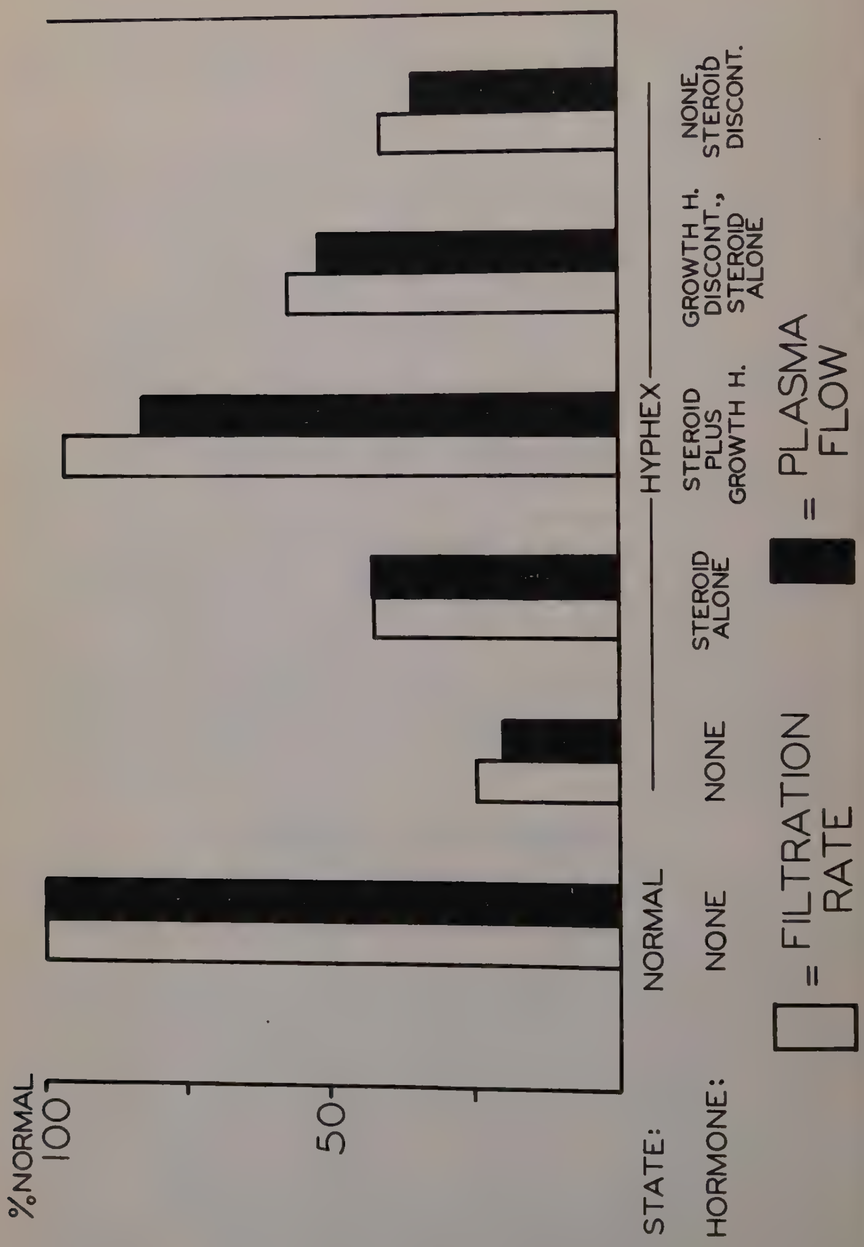

FIGURE 13 *(For description see facing page)*

Therefore, the differences in the above responses of these animals must be due to an anterior pituitary factor(s) other than ACTH in the adrenalectomized and adrenalectomized-gonadectomized dogs that has a role in carbohydrate metabolism. This factor(s) cannot be the gonadotrophins. It exerts its actions in the absence of either the adrenal or sex steroids.

4. Thyroxine produces only a partial abolition of the insulin hypersensitivity of the hypophysectomized dog in dosages which induced hyperthyroidism. Prolactin also produces only a partial abolition of the insulin hypersensitivity; this does not seem to be due to the ACTH contamination.

The possibility that either thyroxine or prolactin, or both, may be the principal anterior pituitary factor or factors (other than ACTH) in the regulation of carbohydrate metabolism is considered remote.

5. Growth hormone, in proper dosages, diminishes and, on continued administration, abolishes the insulin hypersensitivity of the hypophysectomized dog. Prolonged administration is not possible because of the production of either diabetes, toxic manifestations, or both.

6. Growth hormone also can exert these actions in the adrenalectomized-hypophysectomized dog.

Thus growth hormone exerts a marked anti-insulin action. This cannot be attributed to the ACTH content of growth hormone preparations. Furthermore, the adrenal steroids are not absolutely essential for the growth hormone effects in carbohydrate metabolism.

7. Prolonged administration of a "diabetogenic" growth hormone, in relatively large doses, without the production of diabetes or toxicity is possible when combined with concurrent cortisone or compound F administration.

From the above, it is concluded that growth hormone, most probably, is the anterior pituitary factor, in addition to ACTH, which has an important part in the regulation of carbohydrate metabolism. It inhibits the peripheral utilization of sugar, thereby antagonizing the action of insulin and serving to raise the blood sugar. It is suggested that, in physiological life, it exerts its actions in balance with the adrenocortical steroids.

FIGURE 13. *(see opposite page)* Glomerular filtration rate (GFR) and renal plasma flow (RPF) of hypophysectomized (Hyphex) dogs (a) during the untreated state; (b) during steroid regimens alone (16-40 days); (c) during combined steroid-growth hormone regimens (9-21 days); (d) during steroid regimens alone after discontinuation of growth hormone regimens (12-35 days); and (e) after all hormonal therapy had been discontinued (18-40 days), compared with the renal functions of normal dogs.

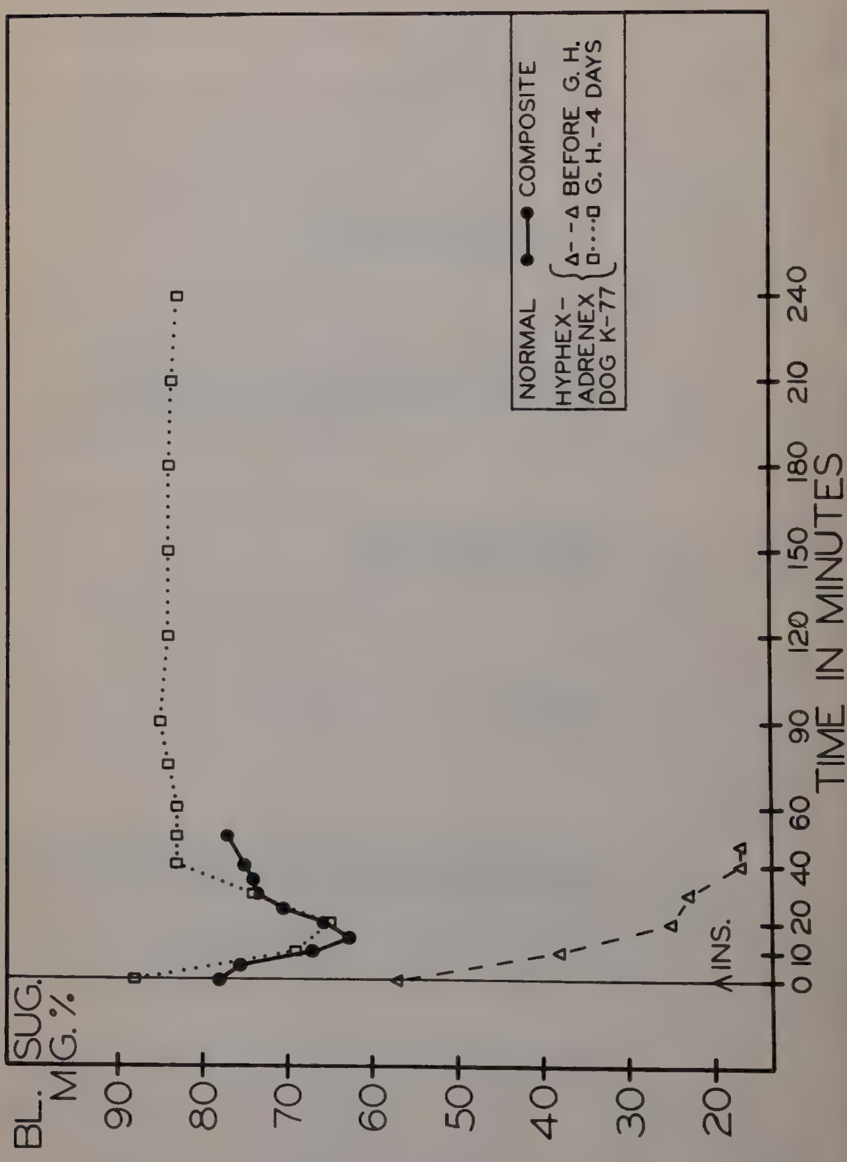

FIGURE 14 *(For description see facing page)*

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FIGURE 14. *(see opposite page)* Blood sugar curve produced by insulin (0.025 u./kg. i.v.) in normal dogs compared with those of hypophysectomized-adrenalectomized (Hyphex-Adrenex) dog K-77 before and during growth hormone regimen. Armour growth hormone No. J21609R, 1 mg./kg./day, given for 4 days.

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